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In vitro Antimicrobial Activity and Phytochemical Analysis of *Jatropha curcas* Roots

[1]O.O. Aiyelaagbe, [2]B.A. Adeniyi, [1]O.F. Fatunsin and [2]B.D. Arimah
[1]Department of Chemistry,
[2]Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria

Abstract: *Jatropha curcas* is an ornamental plant which is also employed to cure various infections in traditional medicine. The hexane, ethyl acetate and methanol extracts of this plant were analysed phytochemically and screened against different microorganisms responsible for various infections especially sexually transmitted diseases. Phytochemical analysis of the extracts revealed the presence of many secondary metabolites including steroids, alkaloids and saponins. The extracts and purified fractions displayed potent antimicrobial activity against the target organisms giving MIC as low as 0.75 μg mL^{-1}. The results confirmed the potency of this plant in treating infections including sexually transmitted infections.

Key words: *Jatropha curcas*, antimicrobial activity, sexually transmitted diseases, vacuum liquid chromatography

INTRODUCTION

Jatropha species belong to the family Euphorbiaceae and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (Chopra *et al*., 1956; Martinez, 1959; Burkill, 1994). *Jatropha curcas* is an ornamental, medicinal and multipurpose shrub which is grown in home gardens in West Africa and cultivated extensively in Asia.

The seeds of *J. curcas* or the expressed oil have been used medicinally as a purgative and as a remedy against syphilis. The oil has been used as a source of fuel, for stimulating hair growth and making candles and soap. The oil burns without smoke and has been employed for street lighting near Rio-de-Janeiro in Brazil. The viscid sap (latex) is employed for cleaning teeth, to cure sores on the tongues of babies and for toothache (Burkill, 1994; Langdon, 1977).

The leaves are utilised extensively in West Africa ethnomedical practise in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Irvine, 1961; Oliver-Bever, 1986). The sap and crushed leaves have also shown anti-parasitic activity (Fagbenro-Beyioku, 1998). The water extract of the branches also strongly inhibited the HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al*., 1999).

The roots are used in decotion as a mouthwash for bleeding gums, toothache, eczema, ringworm, scabies and to cure dysentery and venereal diseases like gonorrhoea. It is also reported that the root methanol extract exhibit anti-diarrhoeal activity in mice through inhibition of prostalglandin biosynthesis and reduction of osmotic pressure (Oliver-Bever, 1986; Mujumdar *et al*., 2001).

Medicinal plants like *J. curcas* have played a major role in the treatment of various diseases including bacterial and fungal infections. The extracts of many *Jatropha* species including *J. curcas* displayed potent cytotoxic, anti-tumor and anti-microbial activities in different assays. The latex of *J. curcas* also showed antibacterial activity against *Staphylococcus aureus* (Thomas, 1989), however the antimicrobial activity of the other parts have not been fully investigated. This study was therefore designed to investigate the antimicrobial activity of this plant especially activity against organisms implicated in sexually transmitted diseases.

MATERIALS AND METHODS

Plant materials: *Jatropha curcas* (Linn) (Euphorbiaceae) was collected from different locations in Ibadan, Nigeria between June 2001 and June 2003. The plants were authenticated at the Forestry Research Institute Ibadan, where voucher specimens were deposited (Herbarium voucher No. FHI 107674). The plant samples were air-dried, separated into the different parts and ground. The dried and ground plant materials: Rootwood (2.25 kg) and rootbark (1.64 kg) were extracted in the soxhlet extractor

Corresponding Author: O.O. Aiyelaagbe, Department of Chemistry, University of Ibadan, Ibadan 200005, Nigeria
Tel: +234-802-341-5316 Fax: +234-2-810-3118/3043

with hexane, ethyl acetate and methanol (5 L each) successively to give the respective extracts which were concentrated *in vacuo*.

Phytochemical analysis: The extracts were subjected to phytochemical screening to detect the presence of some secondary plant metabolites following standard procedures (Sofowora, 1984).

Antimicrobial assay of the extracts: The following microorgamisms were employed in the assay: *Gardnerella vaginalis* (STI/UCH 2305), *Neisseria gonorrhoea*, (ATCC 19424), *Neisseria gonorrhoea* (STI/UCH 029), *Escherichia coli* (STI/UCH 634), *Escherichia coli* (NCTC 9001), *Staphylococcus aureus* (NCTC 6571), *Klebsiella aerogenes* (STI/UCH 024), *Proteus mirabills* (laboratory strain), *Pseudomonas aeruginosa* (NCTC 6750), *Staphylococcus aureus* (NCTC 6571) and *Candida albicans* (STI/UCH 0992).

(Key: ATCC-American Typed Culture Collection; NCTC-National Culture Typed Center; STI-Sexually Transmitted Infection; UCH-University College Hospital, Ibadan).

The following chemotherapeutic agents were used as control: Gentamycin (10 μg mL^{-1}) and Tioconazole (5 μg mL^{-1}).

Determination of antimicrobial activity: The extracts were subjected to antimicrobial assays using the procedures described by Adeniyi *et al*. (1996).

The sensitivity test agar plates and Sabourand and Dextrose agar plates were each seeded with 0.2 mL of a 1:100 dilution of an overnight culture of some bacteria and the fungi, respectively. Mueller-Hinton agar was used for testing the *Neisseria gonorrhoea* strains, *Gardnerella vaginalis* and *Klebsiella aerogenes*. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer of 8 mm diameter was used to make equidistant and uniform wells on the surface of the agar and into different wells were placed 60 μL of the different extracts/fractions re-suspended in 20% DMSO at a final concentration of 200 mg mL^{-1}. The plates were incubated at 37°C for 24 h and at room temperature for 72 h for bacteria and fungi, respectively after which diameter of zones of inhibition were measured. Since each of the extracts were reconstituted in 20% DMSO before being tested, 20% DMSO was included in each plate as a solvent control besides the chemotherapeutic agents included as positive controls. The antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as Means and Standard errors on Means.

Determination of Minimum Inhibitory Concentration (MIC): The MIC of the crude extracts and fractions were determined using broth dilution method in 96 well μL plates. The highest concentration of extracts and fractions tested were 200 and 100 mg mL^{-1}, respectively, dissolved in 10% of dimethyl sulphoxide and serial 2-fold dilutions were made with water as diluent.

Ten microlitres of each test dilution (in Mueller-Hinton broth and Tryptic soya broth for bacteria and fungi, respectively) was added to wells of a 96-well plate and each well was inoculated with 190 μL of a logarithmic phase test culture diluted to 0.5 Macfarland standard which correspond to 10^7 cfu (when plated on Mueller Hinton agar in petri dish) of a logarithmic phase test culture. The petri dish (for control viable count) and the 96-well plates (for monitoring test) were incubated at 37°C for 24 h after which the lowest concentration that showed no visible growth was recorded as the Minimum Inhibitory Concentration (MIC) for each organism (Adeniyi *et al*., 2000).

Fractionation of the extracts: The extracts were subjected to Vacuum Liquid Chromatography (VLC) to fractionate them. Five grams of each extract was dissolved in chloroform/other suitable solvent and preadsorbed on TLC-grade silica gel (Merck Kieselgel 60 G). The preadsorbed sample was allowed to dry and made into fine powder. The sintered glass funnel (porosity 3) used for the VLC was loaded with silica gel under vacuum ensuring that it was well compacted and uniformly spread. A non-polar solvent (usually hexane) was run through the column under vacuum. Then, the preadsorbed sample was evenly spread on the silica gel. Suction was applied to compress the sample to the silica gel and cotton wool was used to cover the surface to prevent disturbance during the course of the experiment. The column was eluted with 100 mL of different solvent systems of increasing polarity starting with hexane. Ethyl acetate, chloroform and methanol were also used. The concentrated fractions were subjected to TLC and similar fractions were pooled together.

The fractions were also subjected to antimicrobial assay.

RESULTS AND DISCUSSION

The yield of the respective extracts and the results of the phytochemical screening are presented in Table 1.

The extracts showed strong antimicrobial activity against the test organisms at a concentration of 200 mg mL^{-1} which is equivalent to 12 mg/well (Table 2).

The pooled fractions from the VLC of the extracts were also screened against some of the micro-orgamisms

Table 1: Yield and result of phytochemical screening of *J. curcas* root extracts

		Secondary metabolites				
Extracts[a]	Percentage yield (w/w)	Steroids	Alkaloids	Saponins	Tannins	Glycosides
JCRWH	0.38	++	+	-	+	-
JCRWE	0.41	-	+	-	+	-
JCRWM	0.79	++	+	+	+	+
JCRBH	1.02	-	+	+	+	+
JCRBE	1.07	++	+	+	+	-
JCRBM	4.13	++	++	++	++	+

JCRWH-Hexane extract of *J. curcas* root wood, JCRWE-Ethyl acetate extract of *J. curcas* root wood; JCRWM-Methanol extract of *J. curcas* root wood JCRBH-Hexane extract of *J. curcas* root bark; JCRBE-Ethyl acetate extract of *J. curcas* root bark; JCRBM-Methanol extract of *J. curcas* root bark; + = Slightly Positive; ++ = Positive; - = Negative

Table 2: *In vitro* antimicrobial activity of the extracts of *J. curcas* root against STD organism

	Microorganisms[b]/zone of inhibition[c] (mm)									
Extracts[a]	GV	NG1	NG2	EC1	EC2	KA	PM	PA	SA	CA1
JCRWH	-	15±0.3	-	-	-	-	-	-	15±0.4	15±0.3
JCRWE	-	13±0.4	12±0.4	-	-	11±0.4	-	11±0.4	12±0.3	15±0.2
JCRWM	-	-	-	-	-	14±0.3	14±0.3	12±0.2	-	-
JCRBH	-	20±0.2	-	-	14±0.3	-	-	-	18±0.4	21±0.4
JCRBE	13±0.4	15±0.3	15±0.5	12±0.3	-	11±0.4	13±0.2	-	17±0.5	-
JCRBM	17±0.5	11±0.2	17±0.4	17±0.4	14±0.2	15±0.3	15±0.4	16±0.2	11±0.4	-
GENT.	24±0.5	22±0.5	22±0.3	15±0.4	14±0.3	12±0.3	16±0.3	R	18±0.3	NT
TIOC.	NT	NT	NT	NT	NT	NT	NT	NT	NT	19±0.3
DMSO	-	-	-	-	-	-	-	-	-	-

JCRWH-Hexane extract of *J. curcas* root wood; JCRWE-Ethyl acetate extract of *J. curcas* root wood; JCRWM-Methanol extract of *J. curcas* root wood; JCRBH-Hexane extract of *J. curcas* root bark; JCRBE-Ethyl acetate extract of *J. curcas* root bark; JCRBM-Methanol extract of *J. curcas* root bark; Concentration of Extracts = 200 mg mL^{-1} (12 µg/well); GENT = Gentamycin (10 µg mL^{-1}); TIOC = Tioconazole (5 µg mL^{-1}); [b]Microorganisms: GV-*Gardnerella vaginalis* (STI/UCH 2305); NG1-*Neisseria gonorrhoea* (ATCC 19424); NG2-*Neisseria gonorrhoea* (STI/UCH 029); EC1-*Escherichia coli* (STI/UCH 634); EC2-*Escherichia coli* (NCTC 9001); KA-*Klebsiella aerogenes* (STI/UCH 024); PM-*Proteus mirabilis* (laboratory strain); PA-*Pseudomonas aeruginosa* (NCTC 6750); SA-*Staphylococcus aureus* (NCTC 6571); CA1-*Candida albicans* (STI/UCH 0992); [c]Zone of Inhibition; Zone of inhibition of triplicate result expressed as means and standard error on means; Size of well = 8 mm; - = No zone of inhibition; NT = Not Tested; R = Resistant

Table 3: *In vitro* antimicrobial activity of VLC fractions of *Jatropha curcas root*

	Microorganisms[b]/Zone of Inhibition[c] (mm)						
Fractions[a]	GV	NG1	EC2	PA	SA	CA1	CA2
JCRWH1	17±0.3	NT	11±0.4	-	11±0.3	-	-
JCRWH2	16±0.4	NT	16±0.4	-	18±0.4	-	-
JCRWE1	15±0.3	NT	20±0.3	-	25±0.3	17±0.3	10±0.2
JCRWE2	18±0.3	NT	24±0.3	-	21±0.2	20±0.4	12±0.4
JCRWE3	-	NT	19±0.4	-	20±0.3	24±0.4	14±0.2
JCRWE4	-	NT	15±0.3	-	12±0.2	24±0.2	12±0.4
JCRWM1	-	NT	15±0.4	16±0.2	16±0.4	-	10±0.2
JCRWM2	-	NT	16±0.2	-	19±0.3	16±0.4	12±0.4
JCRWM3	14±0.2	NT	-	-	11±0.4	-	-
JCRWM4	18±0.2	NT	-	-	-	-	-
JCRBH1	-	21±0.3	25±0.3	-	15±0.3	11±0.3	19±0.3
JCRBH2	18±0.2	21±0.3	25±0.3	-	18±0.3	13±0.3	20±0.4
JCRBH3	27±0.3	16±0.2	24±0.4	12±0.4	15±0.3	-	-
JCRBH4	26±0.4	20±0.4	24±0.4	-	17±0.4	-	-
JCRBE1	27±0.4	20±0.2	22±0.2	-	12±0.3	-	-
JCRBE2	14±0.2	14±0.4	15±0.3	-	16±0.4	-	14±0.2
JCRBM1	15±0.3	NT	16±0.2	-	17±0.3	-	10±0.2
JCRBM2	-	NT	-	-	14±0.2	-	-
DMSO	-	-	-	-	-	-	-
GENT.	25±0.4	25±0.2	15±0.3	R	18±0.4	NT	NT
TIOC.	NT	NT	NT	NT	NT	20±0.4	I8±0.2

[a]Fractions: JCRWH (1-2)-Pooled VLC fractions of Hexane extract of *J. curcas* root wood; JCRWE (1-4)-Pooled VLC fractions of Ethyl acetate extract; of *J. curcas* root wood; JCRWM (1-4)-Pooled VLC fractions of Methanol extract of *J. curcas* root wood; JCRBH (1-4)-Pooled VLC fractions of Hexane extract of *J. curcas* root bark; JCRBE (1-2)-Pooled VLC fractions of Ethyl acetate extract of *J. curcas* root bark; JCRBM (1-2)-Pooled VLC fractions of Methanol extract of *J. curcas* root bark; Concentration of Fractions = 200 mg mL^{-1} (12 µg/well); GENT = Gentamycin (10 µg mL^{-1}); TIOC = Tioconazole (5 µg mL^{-1}) [b]Microorganisms: GV-*Gardnerella vaginalis* (STI/UCH 2305); NG1-*Neisseria gonorrhoea* (ATCC 19424); EC2-*Escherichia coli* (NCTC 9001); PA-*Pseudomonas aeruginosa* (NCTC 6750); SA-*Staphylococcus aureus* (NCTC 6571); CA1-*Candida albicans* (STI/UCH 0992); CA2-*Candida albicans* (STI/UCH 031); [c]Zone of Inhibition: Zone of inhibition of triplicate result expressed as means and standard error on means Size of well = 8 mm; - = No zone of inhibition; NT = Not Tested; R = Resistant

Table 4: Minimum inhibitory concentration of VLC Fractions of *Jatropha curcas root*

	Microorganisms[b]/MIC[c] ($\mu g\ mL^{-1}$)						
Fractions[a]	GV	NG1	EC2	PA	SA	CA1	CA2
JCRWH1	6.25	NT	12.5	>12.5	12.5	>12.5	12.5
JCRWH2	6.25	NT	-	>12.5	3.12	>12.5	12.5
JCRWE1	6.25	NT	3.12	>12.5	1.56	6.25	12.5
JCRWE2	6.25	NT	3.12	>12.5	3.12	6.25	12.5
JCRWE3	>12.5	NT	3.12	>12.5	3.12	6.25	6.25
JCRWE4	>12.5	NT	6.25	>12.5	6.25	3.12	12.5
JCRWM1	>12.5	NT	6.25	6.25	6.25	6.25	12.5
JCRWM2	>12.5	NT	6.25	>12.5	3.12	3.12	12.5
JCRWM3	6.25	NT	>12.5	>12.5	12.5	>12.5	>12.5
JCRWM4	6.25	NT	>12.5	>12.5	>12.5	>12.5	>12.5
JCRBH1	>12.5	0.75	0.75	>12.5	3.12	6.25	6.25
JCRBH2	1.56	0.75	0.75	>12.5	6.25	6.25	6.25
JCRBH3	0.75	3.12	0.75	12.5	6.25	>12.5	>12.5
JCRBH4	1.56	1.56	1.56	>12.5	1.56	>12.5	>12.5
JCRBE1	1.56	1.56	1.56	>12.5	3.12	>12.5	>12.5
JCRBE2	12.5	12.5	3.12	>12.5	3.12	>12.5	6.25
JCRBM1	6.25	NT	3.12	>12.5	3.12	>12.5	12.5
JCRBM2	>12.5	NT	>12.5	>12.5	12.5	>12.5	12.5

[a]Fractions: As in Table 3; [b]Microorganisms: As in Table 3; [c]MIC-Minimum Inhibitory Concentration ($\mu g\ mL^{-1}$); - = No Activity; NT = Not Tested

used previously (Table 3). Most of the fractions showed potent activity against the micro-organisms, the activity being higher in most cases than the crude extracts. The results are shown in Table 4.

All the extracts except the ethyl acetate extract of the root wood (JCRWE) and hexane extract of the root bark (JCRBH) are rich in steroids, which are common constituents of plants. Alkaloids and tannins are also present in all the root wood and root bark extracts (Table 1), which is in agreement with a previous report in literature (Rahila *et al*., 1994). All the methanol extracts are richer in these metabolites than the hexane and ethyl acetate extracts which may be due to the high polarity of methanol, hence its ability to extract more components.

It is noteworthy that the methanol extract of the root bark showed potent broad-spectrum activity against all the microorganisms except *Candida albicans*. It is even more active than Gentamycin, with respect to *Escherichia coli*, *Klebsiella aerogenes* and *Pseudomonas aeruginosa*. It is equally notable that JCRWE, JCRWM and JCRBM were active against the strain of *P. aeruginosa* used, which was resistant to gentamycin. Three of the extracts (JCRWH, JCRWE, JCRBH) also showed strong activity against the clinical strain of *Candida albicans* used (Table 2). The hexane extract of the root bark (JCRBH) is the most potent against *Candida albicans* and the most active against *Neisseria gonorrhoea* and *Staphylococcus aureus*.

The activity of the VLC fractions was more pronounced than that of the crude extracts as expected (Table 3 and 4). The root bark hexane and ethyl acetate fractions were the most potent with activities comparable to that of gentamycin and tioconazole and even higher in some cases.

Jatropha curcas roots are used for treating eczema, ringworm and gonorrhoea and these diseases are caused by fungi and bacteria infections. Previous work (Dekker *et al*., 1986; Aiyelaagbe *et al*., 2000; Aiyelaagbe, 2001) has shown that many *Jatropha* species possess antimicrobial activity, but this is the first report of antimicrobial activity of the extracts of *Jatropha curcas* against bacteria and fungi STD microorganisms. The antimicrobial activity of these extracts explains the many uses of the plant in ethnomedicine.

CONCLUSIONS

This study has revealed the presence of many secondary metabolites in the roots of *J. curcas*. It has further confirmed that the plant extracts could be used for the treatment of various infections including sexually transmitted infections. The results lend credence to the folkloric use of this plant in treating microbial infections and shows that *J. curcas* could be exploited for new potent antibiotics.

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